cancers

MDPI

*Supplementary Materials*

# The Antidiabetic Agent Acarbose Improves Anti-PD-1 and Rapamycin Efficacy in Preclinical Renal Cancer

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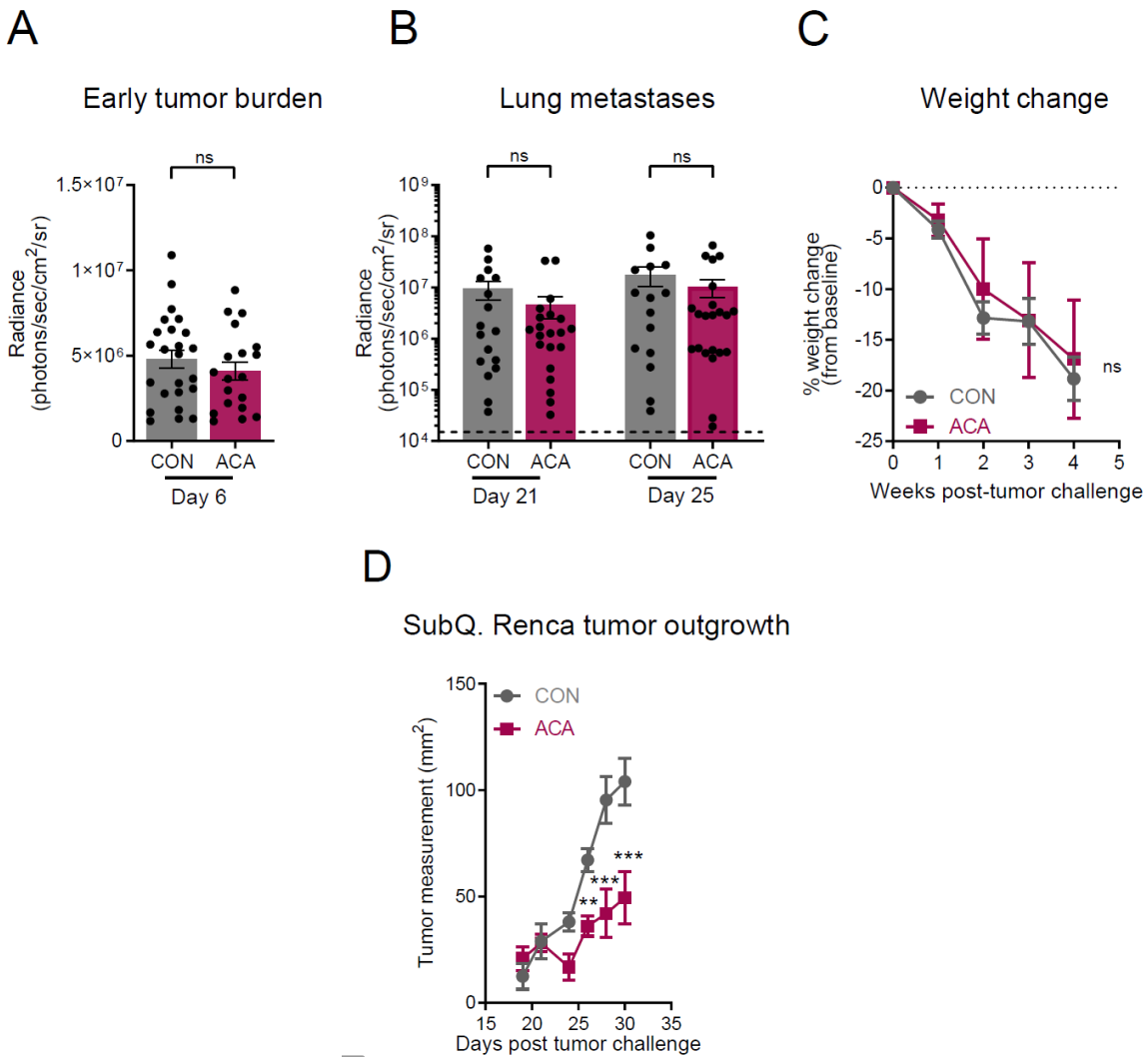


**Figure S1.** Acarbose does not alter early tumor burden, lung metastases, or weight loss. (**A**) BLI quantification of day 6 primary renal tumor burdens. (**B**) Day 21 BLI quantification of day 21 and 25 excised metastatic lungs. Dotted black line represents the average BLI of tumor-free lungs. (**C**) Percent weight change over time versus baseline body weights. (**D**) Tumor areas measured by caliper over time of Renca tumor cells injected subcutaneously. Data from individual mice from at least 3 experiments are shown in **A** and **B.** Data in **C** and **D** are summary data from 2 experiments with $n$ = 6-24 mice per group. Data are presented as means ± SEM. Statistical differences were determined using nonparametric Mann-Whitney tests in **A** and **B**. Statistical differences in **C** and **D** were determined through two-way repeated measures or mixed models ANOVA (ns = not significant; * = $p < 0.01$; *** = $p < 0.001$). CON = Control diet; ACA = Acarbose-containing diet.

A

| Diet | Composition |
|---|---|
| 5LG6 Control diet (5LG6 CON) | 20.5% kcal protein<br>12.1% kcal fat<br>67.4% kcal carbohydrates |
| 5LG6 Acarbose-containing diet (5LG6+ACA) | 20.5% kcal protein<br>12.1% kcal fat<br>67.4% kcal carbohydrates<br>0.1% Acarbose |

B

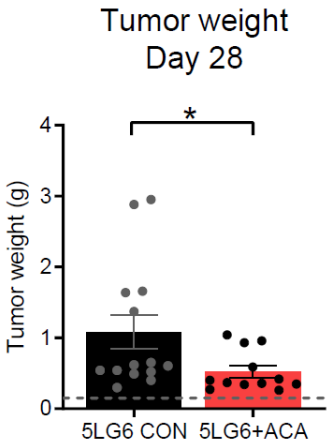


**Figure 2.** Acarbose impairs renal tumor growth in mice fed the 5LG6 diet composition. (**A**) Macronutrient composition percentages in kilocalories (kcal) of 5LG6 CON and 5LG6 + ACA diets. (**B**) Day 28 excised renal tumor weights. Dotted gray line represents the average weight of tumor-free kidneys.Data in **B** are from 2-3 independent experiments and data from individual mice are shown. Data are presented as means ±SEM. Statistical differences were determined using nonparametric Mann-Whitney tests (* = $p < 0.05$). 5LG6 CON = 5LG6 Control diet; 5LG6 + ACA = 5LG6 Acarbose-containing diet.

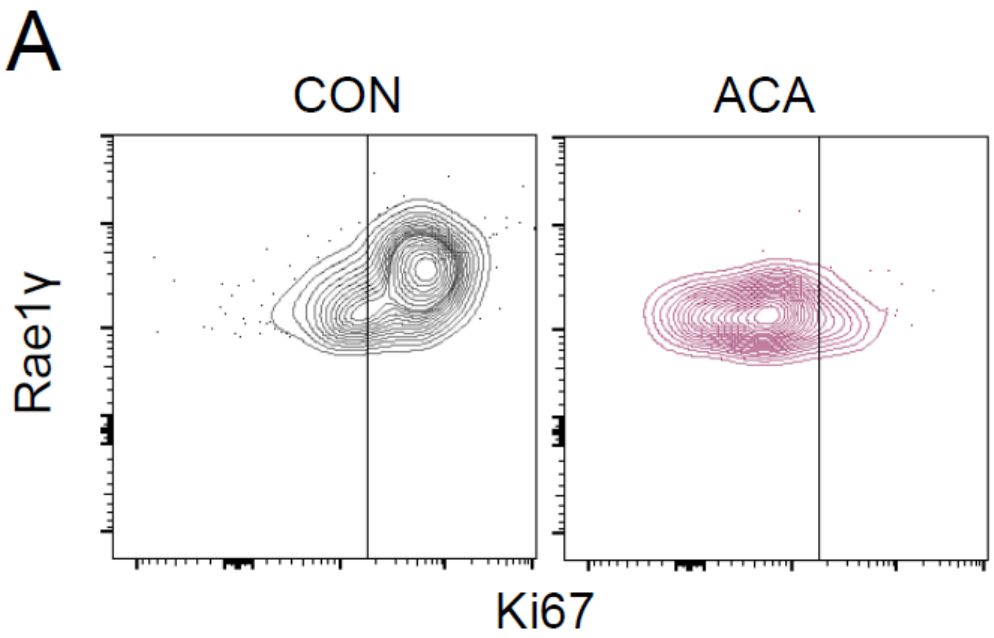


**Figure S3.** Renal tumor cells from day 21 tumors exhibit less Ki67 staining with acarbose. (**A**) Representative Ki67 flow cytometry plots from day 21 renal tumors (*Ancestry: FSC-A vs SSC-A > Single cells > Live > CD45-Rae1γ+*). CON = Control diet; ACA = Acarbose-containing diet.

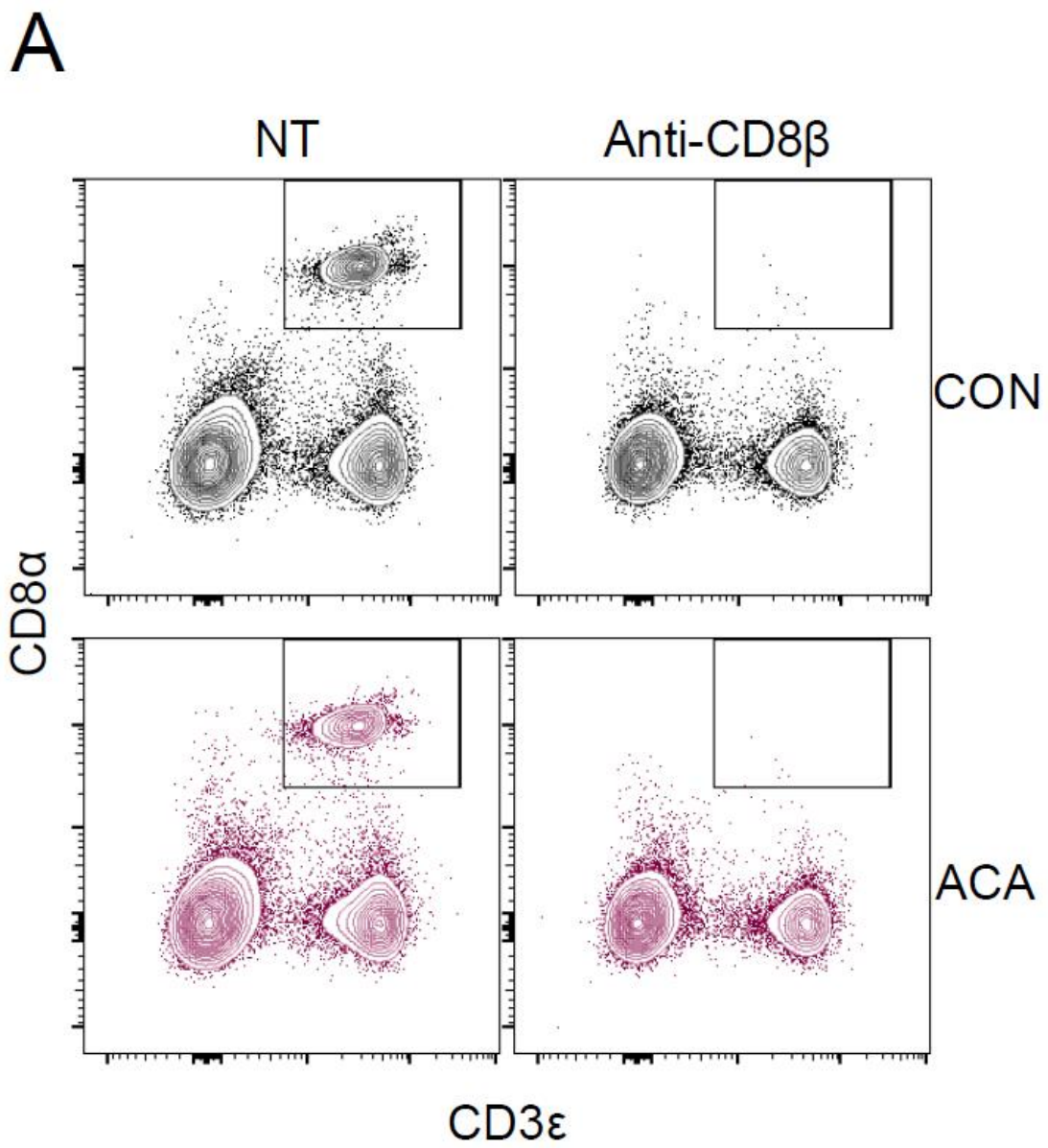


**Figure S4.** Depletion of CD8 T cells in mice on CON and ACA. (**A**) Representative flow cytometry plots from day 21 spleens from CD8-intact mice and following CD8 depletion with anti-CD8β. (*Ancestry: FSC-A vs SSC-A > Single cells > Live*). CON = Control diet; ACA= Acarbose-containing diet; NT = no treatment.

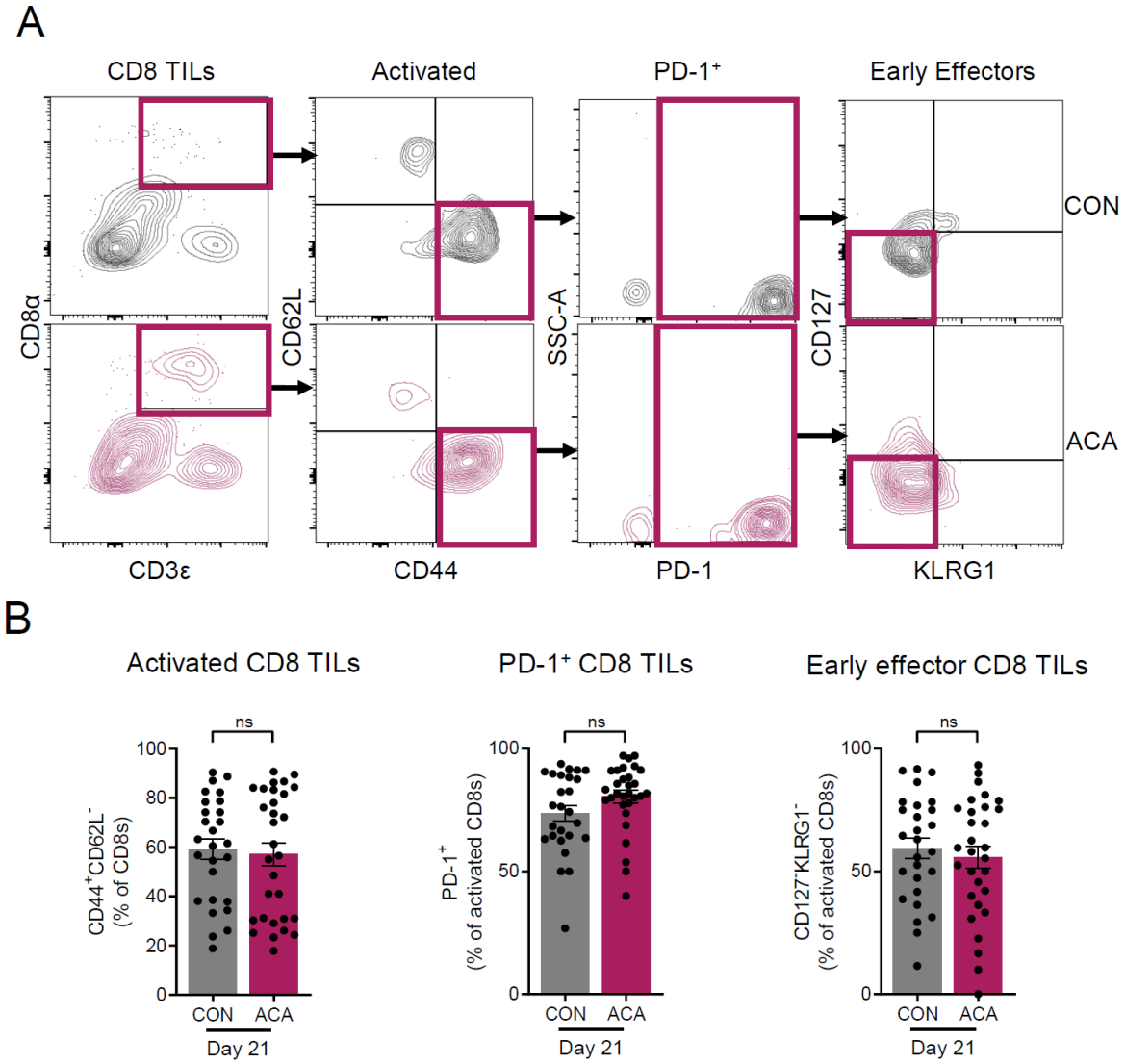


**Figure S5.** CD8 TILs from mice on CON and ACA exhibit similar phenotypes. (**A**) Representative gating strategies from day 21 renal tumors to define activated ($CD44^+CD62L^-$), PD-1+, and early effector populations ($CD127^-KLRG1^-$). (**B**) Pooled data from activated, PD-1+ and early effector populations from day 21 renal tumors. Data from individual mice from at least 3 experiments are shown**.** Data are presented as means ± SEM. Statistical differences were determined using t-tests or nonparametric Mann-Whitney tests. CON = Control diet; ACA= Acarbose-containing diet. ns = not significant.

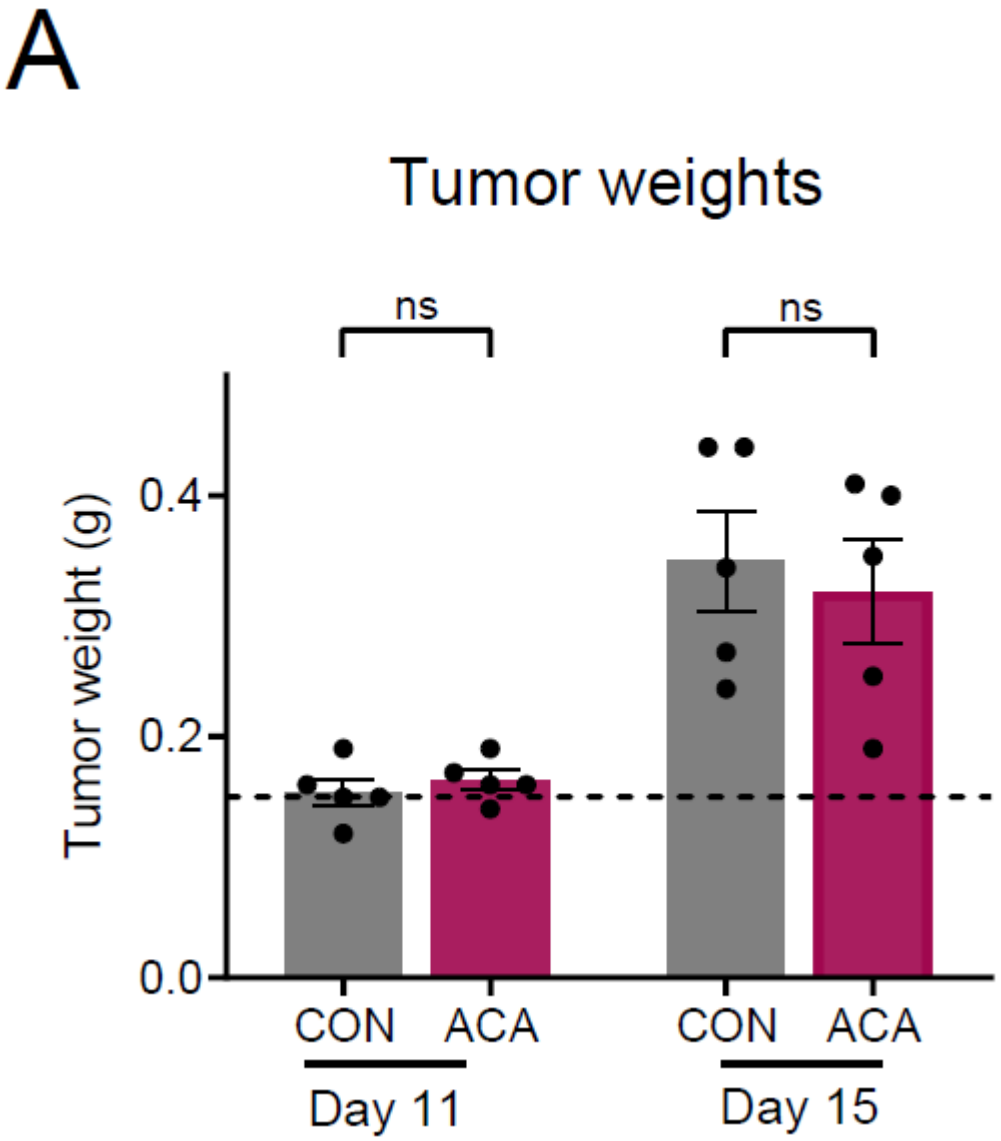


**Figure S6.** Renal tumor weights are similar with ACA in the early stages of tumor growth. (**A**) Excised renal tumor weights from days 11 and 15. CON = Control diet; ACA = Acarbose-containing diet. Data from individual mice from at least 1 experiment per time point are shown. Data are presented as means ± SEM. Statistical differences were determined using nonparametric Mann-Whitney tests (ns = not significant). CON = Control diet; ACA= Acarbose-containing diet.

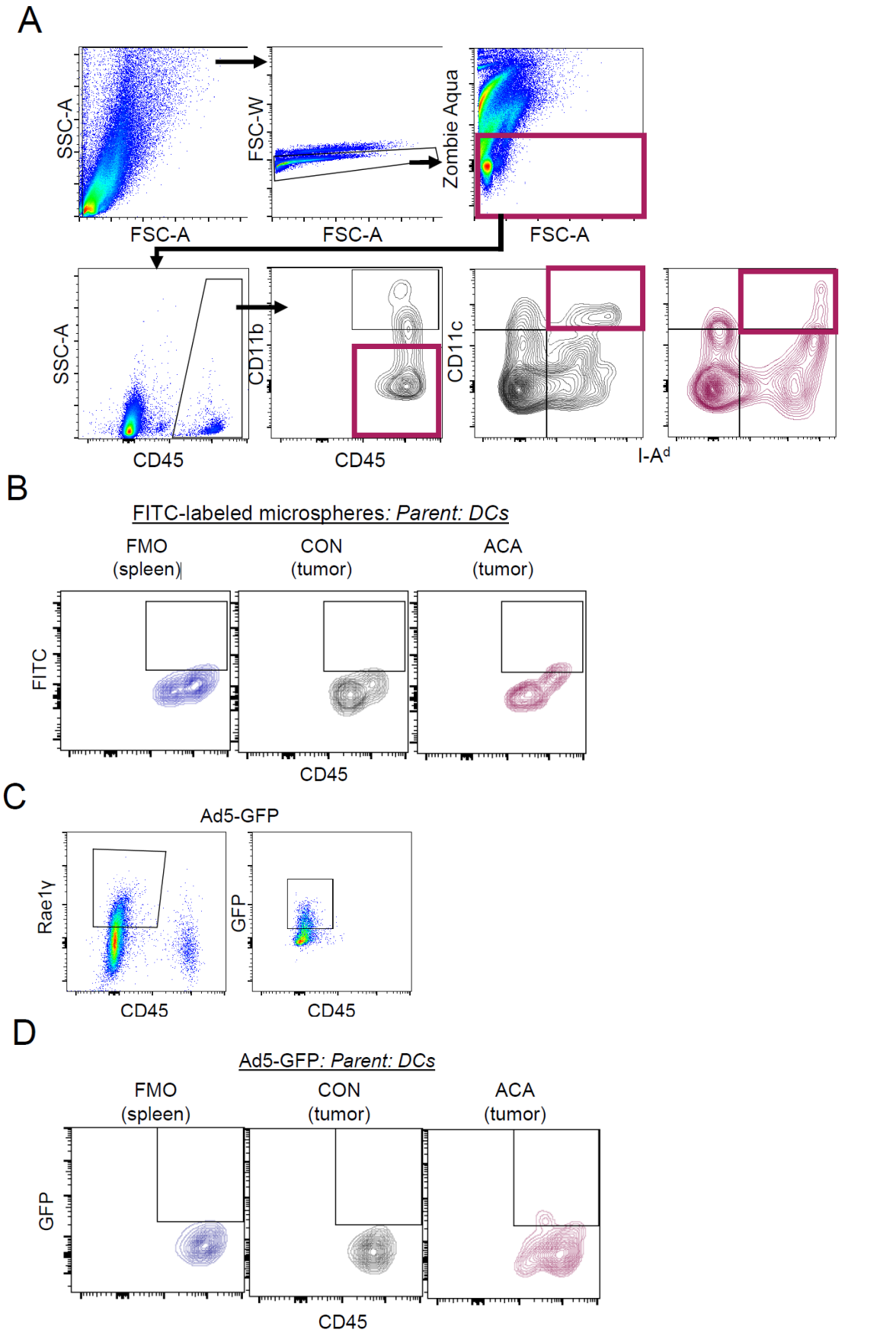


**Figure S7.** Defining dendritic cell subsets and GFP expression in day 11 renal tumors (**A**) Gating strategy from day 11 renal tumors for defining dendritic cells (DCs). (**B**) Intratumoral DC expression of FITC. (**C**) Day 11 tumor cell expression of GFP. (**D**) Intratumoral DC expression of GFP.

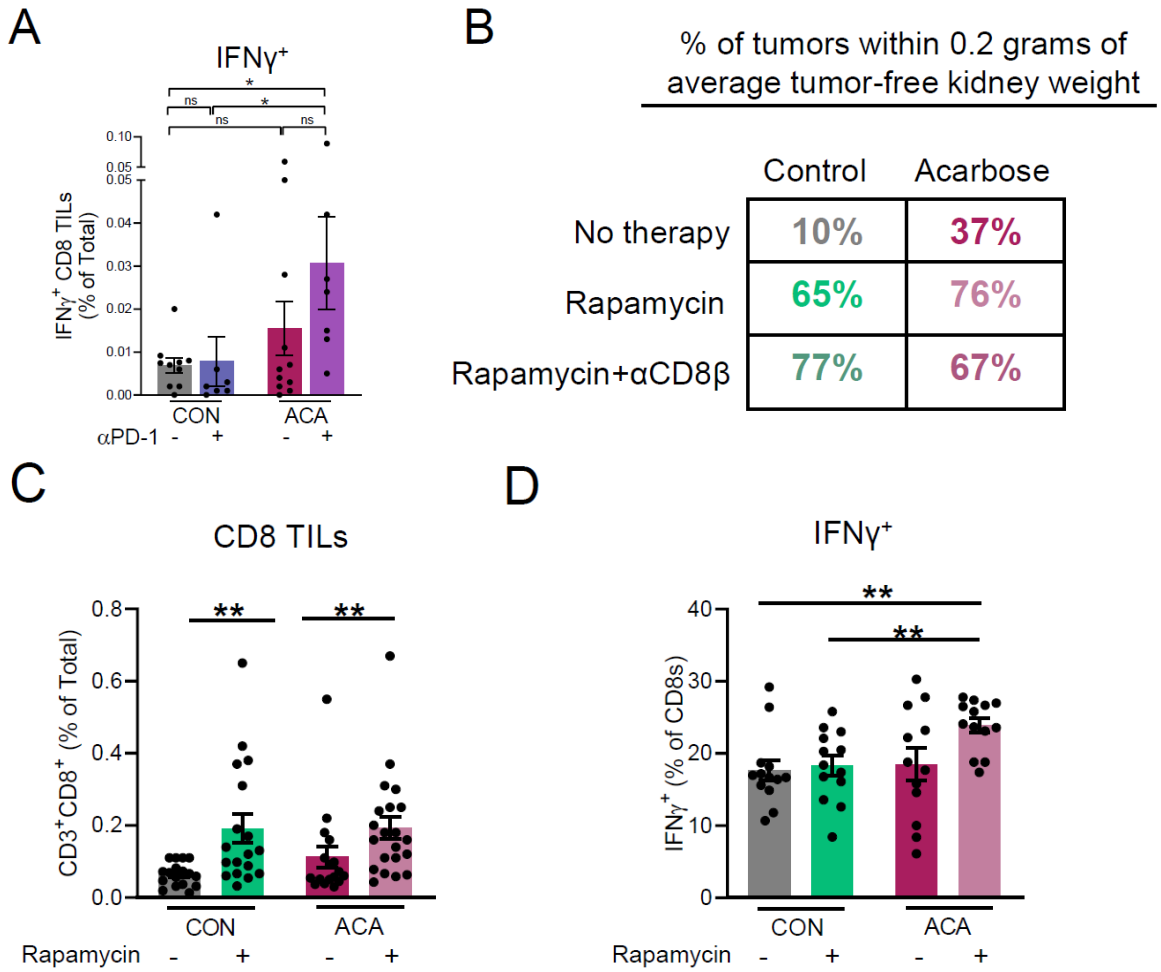


**Figure 8.** Combinatorial therapeutic outcomes with ACA + anti-PD-1 or rapamycin. (**A**) Frequency of IFNγ+CD8 T cells within day 21 renal tumors as a frequency of total cells evaluated (*Ancestry: FSC-A vs SSC-A > Single cells > Live, CD11b-CD11c-dump gate > CD3⁺CD8⁺*). (**B**) Percentages of tumors within 0.2 grams of a tumor-free kidney across all groups from rapamycin experiments. (**C**) Frequency of CD8 T cells within day 21 renal tumors as a frequency of total cells evaluated (*Ancestry: FSC-A vs SSC-A > Single cells > Live, CD11b-CD11c-dump gate*) (**D**) Frequencies CD8 TILs producing of IFNγ⁺ within day 21 renal tumors (*Ancestry: FSC-A vs SSC-A > Single cells > Live, CD11b-CD11c-dump gate > CD3⁺CD8⁺)*. Data from individual mice are shown and are from 2-4 independent experiments. Data are presented as means ± SEM. Statistical differences were determined using one-way ANOVA with Tukey's post hoc test or nonparametric Kruskal-Wallis ANOVA with Dunn's post hoc test. (ns = not significant; *$p < 0.05$, **$p < 0.01$). CON = Control diet; ACA = Acarbose-containing diet.

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